

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re-application of

Perez et al.

Group art Unit: 1638

Serial N° : 10/048,185

Examiner: Robinson K.

Filed: June 17, 2002

For: METHOD FOR OBTAINING ISOGENIC LINES

**DECLARATION UNDER RULE 132**

Hon Commissioner of Patents and Trademarks

WASHINGTON DC. 20231

Sir:

I, Pascual PEREZ, residing at 17, Chemin de la Pradelle Varennes, 63450 Chanonat (France);

Declare and say:

I am citizen of France,

[1]. I am graduated from the University Paul SABATIER (Toulouse, FRANCE), where I got a Master degree of Molecular Biology and Biochemistry in 1981, followed by a Graduate Diploma of Applied Studies (DEA) in Microbiology and Microbial Genetics obtained in 1982 in the same University.

[2]. I am currently working as Laboratory Head Manager and Research Coordinator (Corn Transformation & Functional Genomic Group leader) in the Biogemma laboratory located 24, avenue des Landais 63170 Aubière (France), where my team and I are working in particular on maize transformations methods.

I am named as an inventor of five granted US patents and more than 10 pending US applications.

I am also aware of transformation techniques for species other than maize, being an inventor for a European patent application named "Regeneration and genetic transformation of sugar beet" (EP 517 833).

[3]. I am an inventor of the present patent application and I am aware that the Examiner has rejected the instant claims for an alleged lack of enablement, lack of written description, lack of novelty and lack of inventiveness.

[4]. The Examiner indicates that no lines other than the transformation adapted maize line A188 are described in the specification, making it impossible to perform the invention with another line than A188 and other crops than maize.

I submit that persons skilled in the art, in the field of plant transformations, are aware of which line adapted to transformation to choose for a specific species.

As an example, documents Damgarrrd et al (1997), Gurel et al (1999), Davies et al (1991), Stewart et al (1996), Bordas et al (1997) describe different lines adapted for transformation for different type of species.

As for maize, the instant specification mentions the use of Hi-II as an alternative, said use having been demonstrated by others (see US 20030046724 or US20040194161).

Furthermore, I submit that methods of making hybrids (crossing two lines) are well known in the art and work for any species, thus making it possible to transform any hybrid formed from a cross between a line of interest and a line adapted to transformation.

[5]. The Examiner indicates that the specification does not describe methods for analyzing and comparing genomes of transformed plants.

I submit that these methods are well known in the art, and that the person skilled in the art can discriminate the parental origin of any chromosome in a hybrid, or after crosses.

Indeed, document Welsh et al (1990) describes the AP-PCR method and demonstrates that it is possible to discriminate genomes event without any prior sequence information (abstract). Welsh et al. also indicate page 7216, column 2, 5th paragraph) that "AP-PCR will work with most genome and species" (having tested it on rice, maize and human genomes, where it works).

Furthermore, US 5,332,408 relates to sequences that are specific of particular plant species, subspecies or variety (see column 4, lines 31-35), and gives methods to isolate such sequences. Claims are broad and not restricted to specific species, subspecies or variety. This

document demonstrates that methods of isolation of probes specific of any particular plant species, subspecies or variety were well known in the art at the time the patent application was filed.

[6]. The Examiner considers that the method described in the instant application would not work with other crops than maize. As demonstrated above, people skilled in the art can easily perform the method of the invention with any crop, choosing a line adapted to transformation, making an hybrid with a line of interest, transforming this hybrid, and analyzing the transformants to select those where the transgene has integrated in the genome of line of interest.

[7]. I can agree with the Examiner's view that plants of different species will vary in their genotypic composition, and that there is genotypic variation within a species. I can also agree that transformation is genotype dependent. This is precisely why the invention will work and make it possible to have true isotransgenic lines.

As indicated in the instant specification, it is indeed difficult to transform any genotype. The invention solves this problem by transforming a hybrid between a line adapted for transformation and the line of interest. As indicated above, the person skilled in the art knows such favorable genotypes for transformation for any species.

From a scientific point of view and without being bound by this theory, I would suggest that the hybrid would join the "transformation-favorable genotype" to the "line of interest genotype", thus improving the transformation rate, and making it possible to obtain transformants as described in the examples.

[8]. In conclusion, I submit that the person skilled in the art, wishing to transform a specific line of interest of a given species, would know how to choose a line adapted to transformation in order to make a hybrid between said specific line and said line adapted to transformation.

The person skilled in the art, using the available literature, would also know how to transform said hybrid, how to discriminate the genomes of said specific line and said line adapted to transformation, and how to perform backcrosses in order to obtain isotransgenic lines.

[9]. Thus, it is my belief that the person skilled in the art can perform the method as claimed and obtain the claimed isotrangenec lines for virtually any species.

[10]. The method that is claimed is a generic method. I believe that this method is described and understandable for a person skilled in the art, as soon as all steps for performing the method are enunciated, and are known in the art.

Furthermore, it is obvious to me that the claimed isogenic lines are plant lines containing a transgene comprising only the T-DNA and said transgene. It is clear to me that this describes perfectly the lines, and that a person skilled in the art would recognize what it is.

[11]. I have studied document Ragot et al, cited by the Examiner, who indicates that the corn lines of Ragot et al. can theoretically be identical to the claimed isotransgenic lines of the invention. It is my belief that Ragot et al. actually relate to the problem solved by the method of the instant claims (presence of a genetic drag due to the integration of the transgene in a line adapted for transformation, that is distinct from the line of interest). It is also my knowledge that elimination of all the regions flanking the T-DNA and the transgene and originating from the line suited for transformation, only by backcrossing is virtually impossible, as the probability of a recombination event making it possible is near zero.

Indeed, 1 centimorgan is equivalent, on average, to about 1,5 million base pairs. Sequences being spaced by 1 centimorgan of distance have a 1% probability of being separated by a recombination event.

Transgenic genes + T-DNA usually are about 2000-3000 bases, which is 0,0015-0,002 centimorgan. Thus, the probability to obtain a true isotransgenic line as described and claimed in the instant application is 0,0015-0,002 %, which is virtually impossible to put into practice, at least without undue burden.

Ragot et al. mention production of "near isogenic lines" (page 45, introduction 2nd §), and perform introgression of a transgene from a Lancaster maize line within a Stiff Stalk line (page 46, plant material). These are two different lines.

Figure 1-d clearly demonstrates that the transgene locus remains heterozygous after 4 backcrosses (chromosome 1), while all other chromosomes have been homozygous since the third backcross (see also figure 1-c).

As indicated above, these figures 1-c and 1-d indicate that the limiting step is to obtain the appropriate recombination events around the transgene locus.

Thus Ragot et al. do not describe any true isotransgenic line as compared to a line of interest (which would there be Stiff Stalk), wherein said isotransgenic line only differs from said line of interest by the presence of the T-DNA containing the transgene, since there is still some Lancaster genomic sequences

[12]. The Examiner further indicates that the instant specification does not disclose how many backcrosses are needed to obtain the isotransgenic lines.

It is reminded that backcrosses for introgression of a trait from a line in another line are exemplified in Ragot et al. (thus known in the art) and discussed in the specification of the instant application. Introgression is made possible by virtue of homeologous recombination events at the meiosis stage (see enclosed figures 3-5). If such recombination did not occur, it would be completely impossible to perform such introgression (see figures 1 and 2). In this figure, the brown bars represent chromosomes from the line suited for transformation, whereas the blue bars represent the chromosome from a line of interest, according to the invention. As a measure of clarity, only the pair of chromosome containing the transgene (black line) are represented.

Moreover, US 5,332,408 relates to introgression of a given trait into a line of interest through backcrossing, and the use of molecular markers. This document demonstrates that these methods were largely known in the art and that determining the number of introgression to perform is within the skills of persons in the art.

[13]. The number of backcrosses to perform in order to get an isotransgenic line of the specific line of interest, is easily determined when using molecular markers for assessing presence of the genome of said line adapted to transformation in the progeny obtained from backcrosses.

Backcrosses are eased by the use of the method of the invention (see figures 6-7), as

- 1) since the transformation is performed directly on an hybrid, the transformants already contain 50% of genome of the line of interest, which is one step better than when backcrosses are started from a line completely distinct from said line of interest, as is the case when a line adapted to transformation is used (see figures 3 and 4).
- 2) since the initial transformants are chosen so that transgene is integrated within the chromosome of the specific line, the follow-up of the transgene can be performed by following-up of the chromosome of the specific line bearing it, and does not involve follow-up of recombination events, as usually compulsory with back-cross methods, where the transgene is integrated in chromosomes of a line distinct from said line of interest;

From figure 1-c of Ragot et al. (chromosomes which do not carry the transgene are homozygous from the third backcross), I would actually submit that 3 or 4 backcrosses should be enough to obtain the isotransgenic lines of the invention.

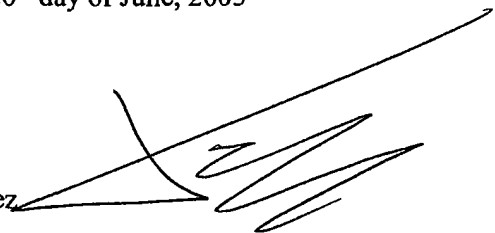
[14]. I have also studied documents Ishida (1996), Does (1991), Hie (1994), Armstrong (1992), and Ragot cited by the Examiner as pertaining to assessment of inventiveness. The problem solved by the invention is the creation of true isotransgenic lines, from lines that are reluctant to transformation. Solving this problem was practically impossible, using conventional methods (transformation of a line suited for transformation, followed by backcrosses), as indicated above. It is my belief that none of these cited documents, either alone or in combination, gives a solution to this problem, nor describes or suggests the step of selecting, among hybrid primary transformants, at least one individual in which said T-DNA has integrated only into the genome of the line of interest, in order to obtain isotransgenic lines.

[15]. I also declare that, to my knowledge, at the priority date, there was no described method in the art to obtain the true isotransgenic lines as claimed in this patent application, and that no true isotransgenic lines had been described in the art. I thus believe that the method and lines as described in the invention are a solution to a problem (linkage drag) that was still pending and not solved when the patent application was filed.

[16]. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true ; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed this 20<sup>th</sup> day of June, 2005

Pascual Perez

A handwritten signature in black ink, consisting of a series of loops and strokes, positioned to the right of the printed name 'Pascual Perez'.

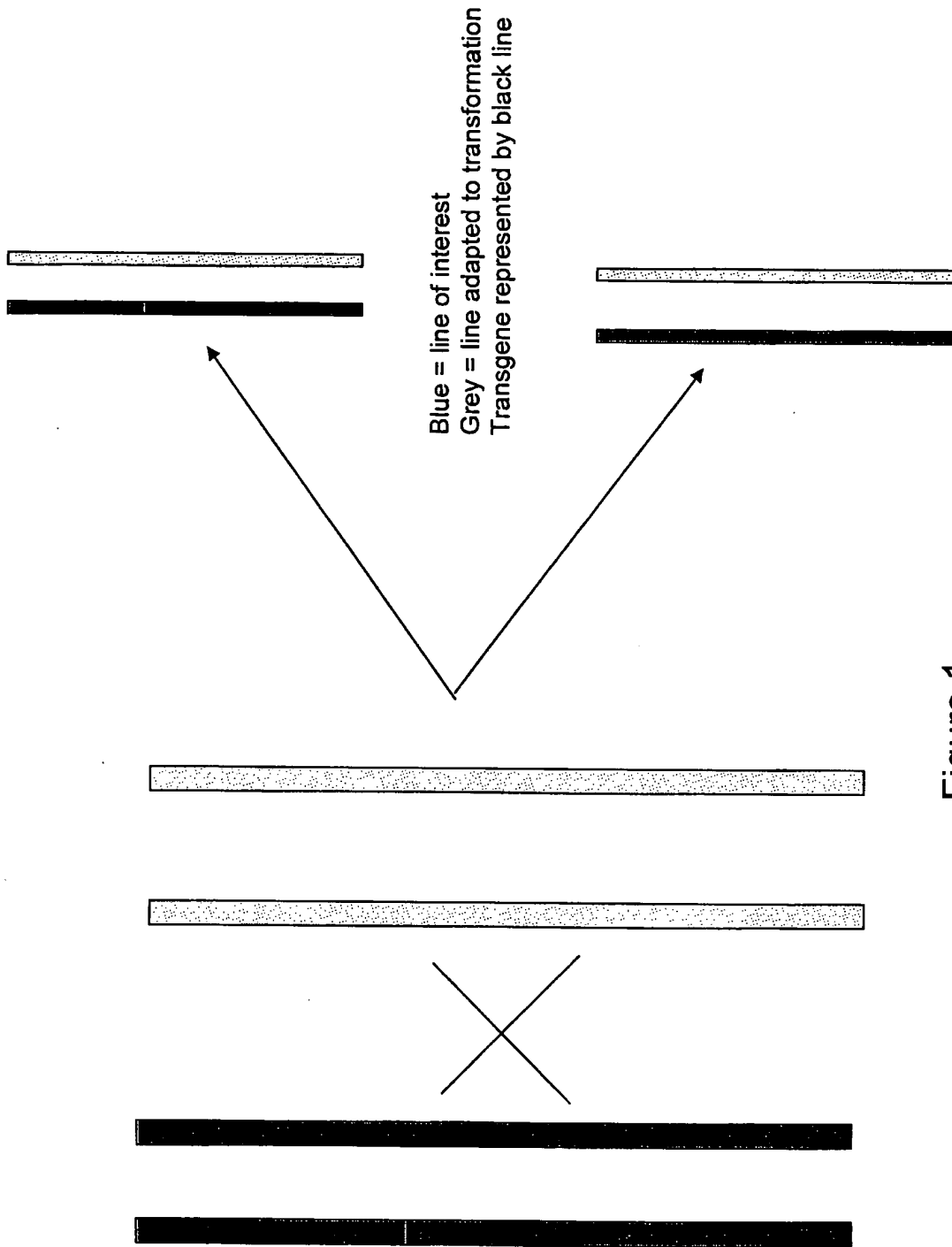


Figure 1  
Crosses without meiotic recombination events

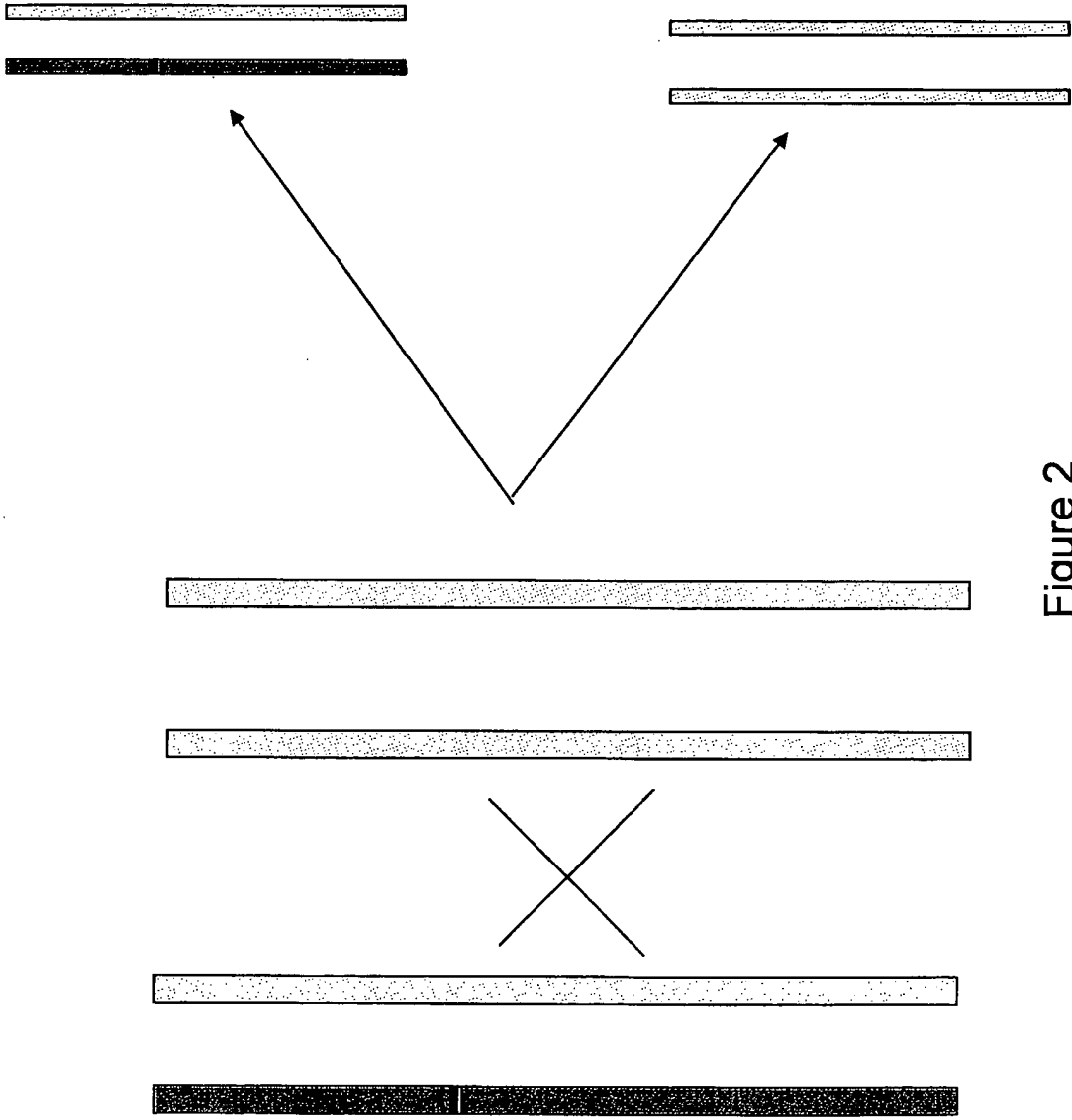


Figure 2  
Crosses without meiotic recombination events



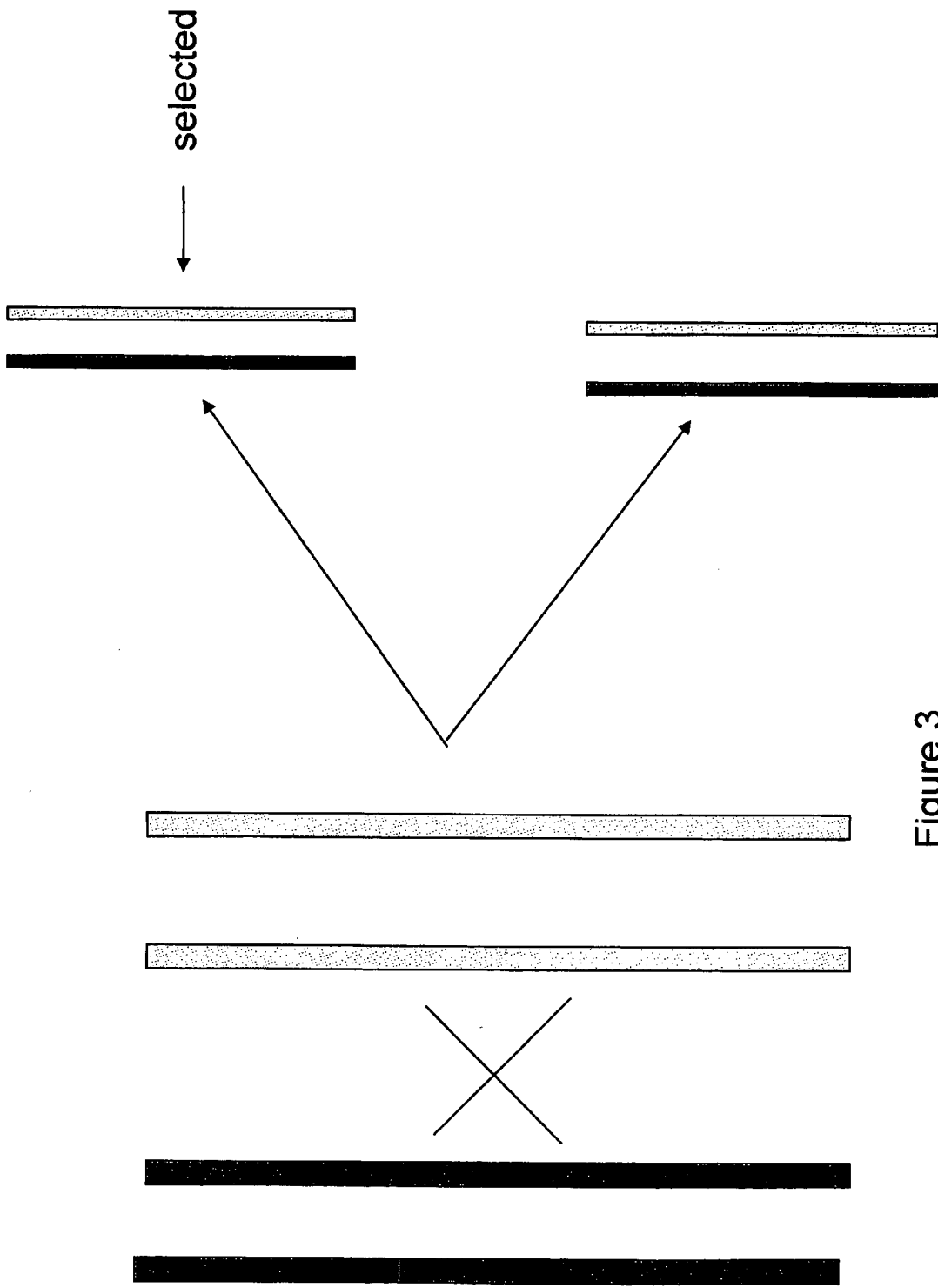


Figure 3  
Method of the prior art

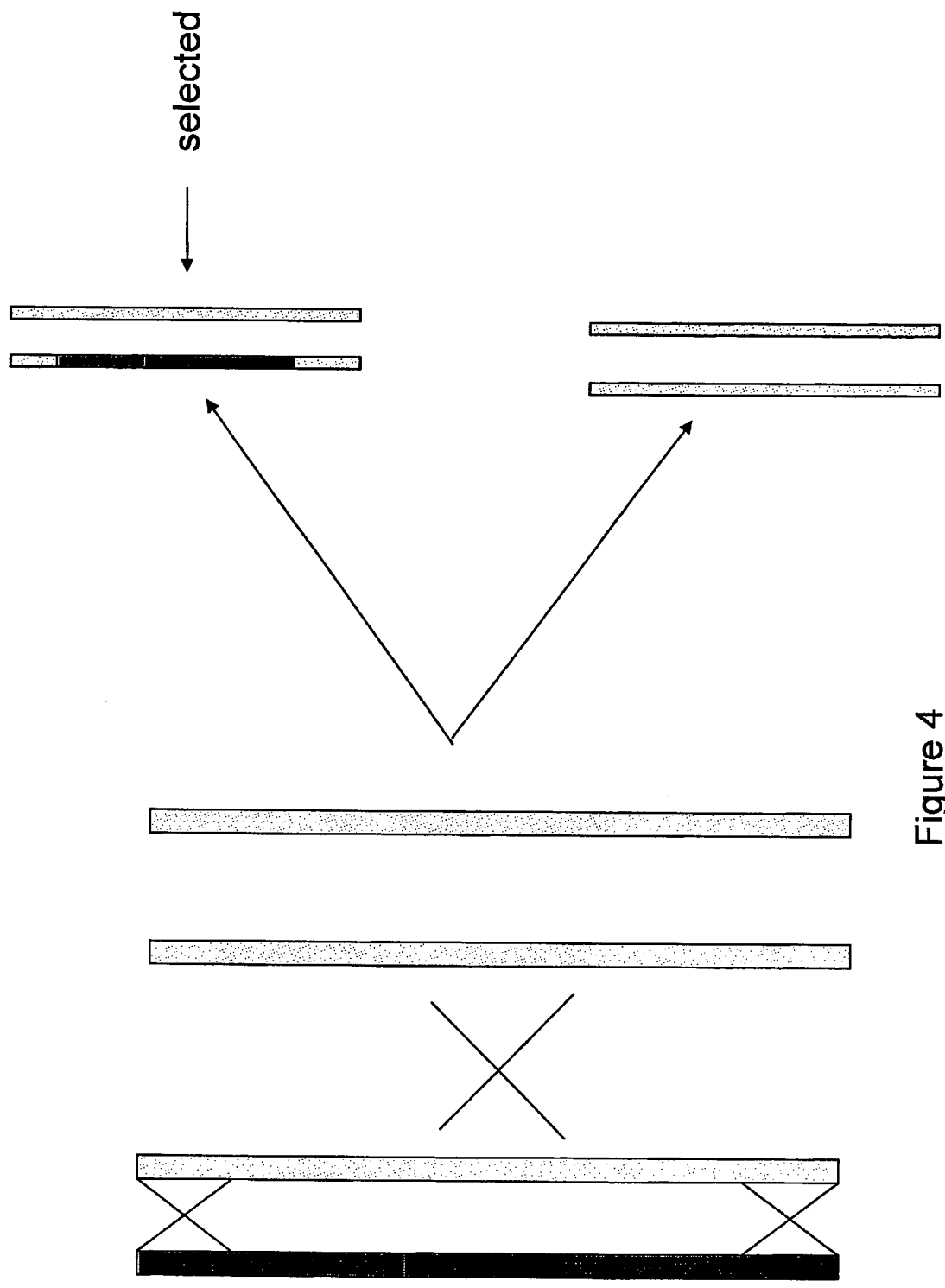


Figure 4  
Method of the prior art

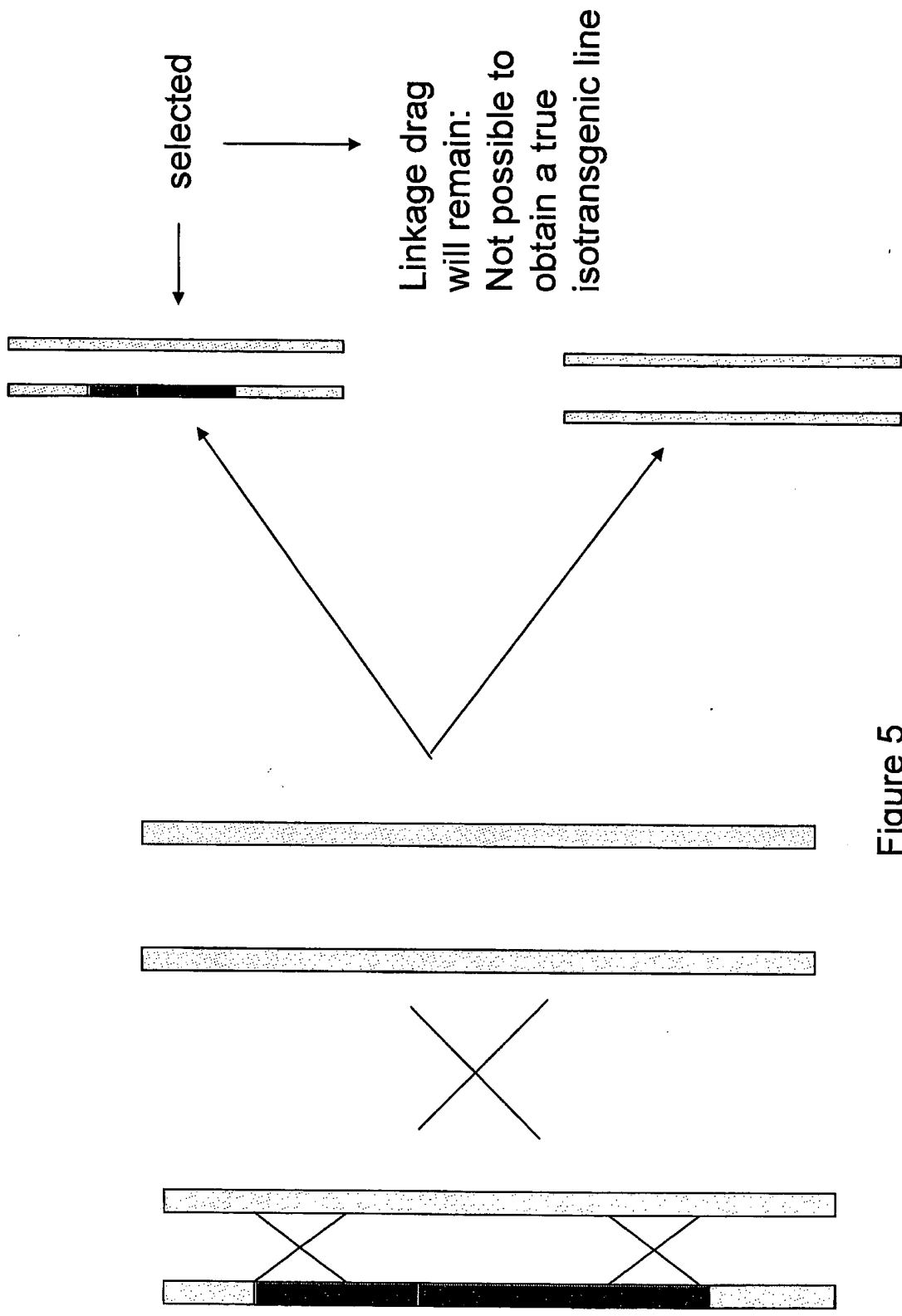


Figure 5  
Method of the prior art

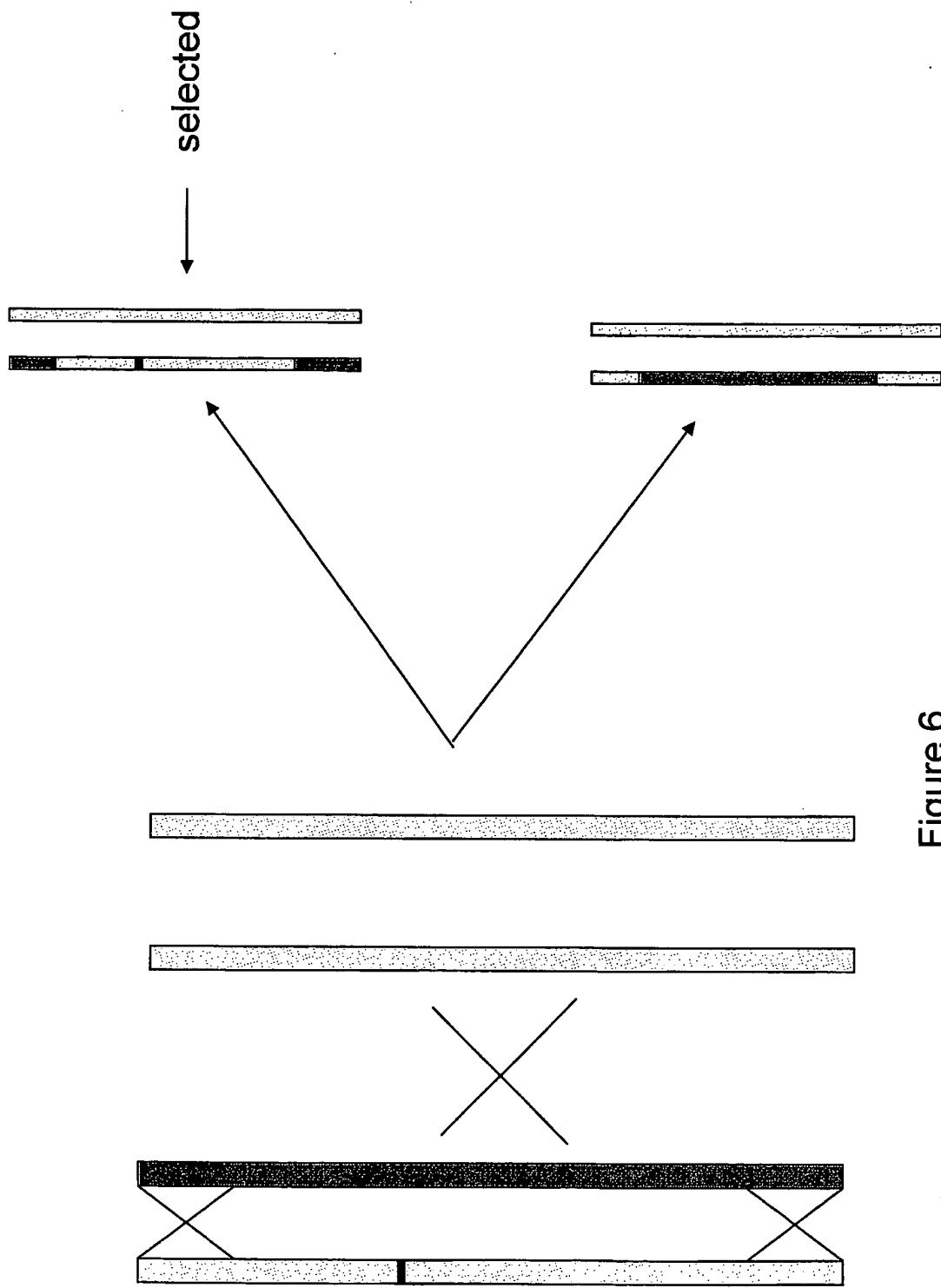


Figure 6  
Method according to the invention

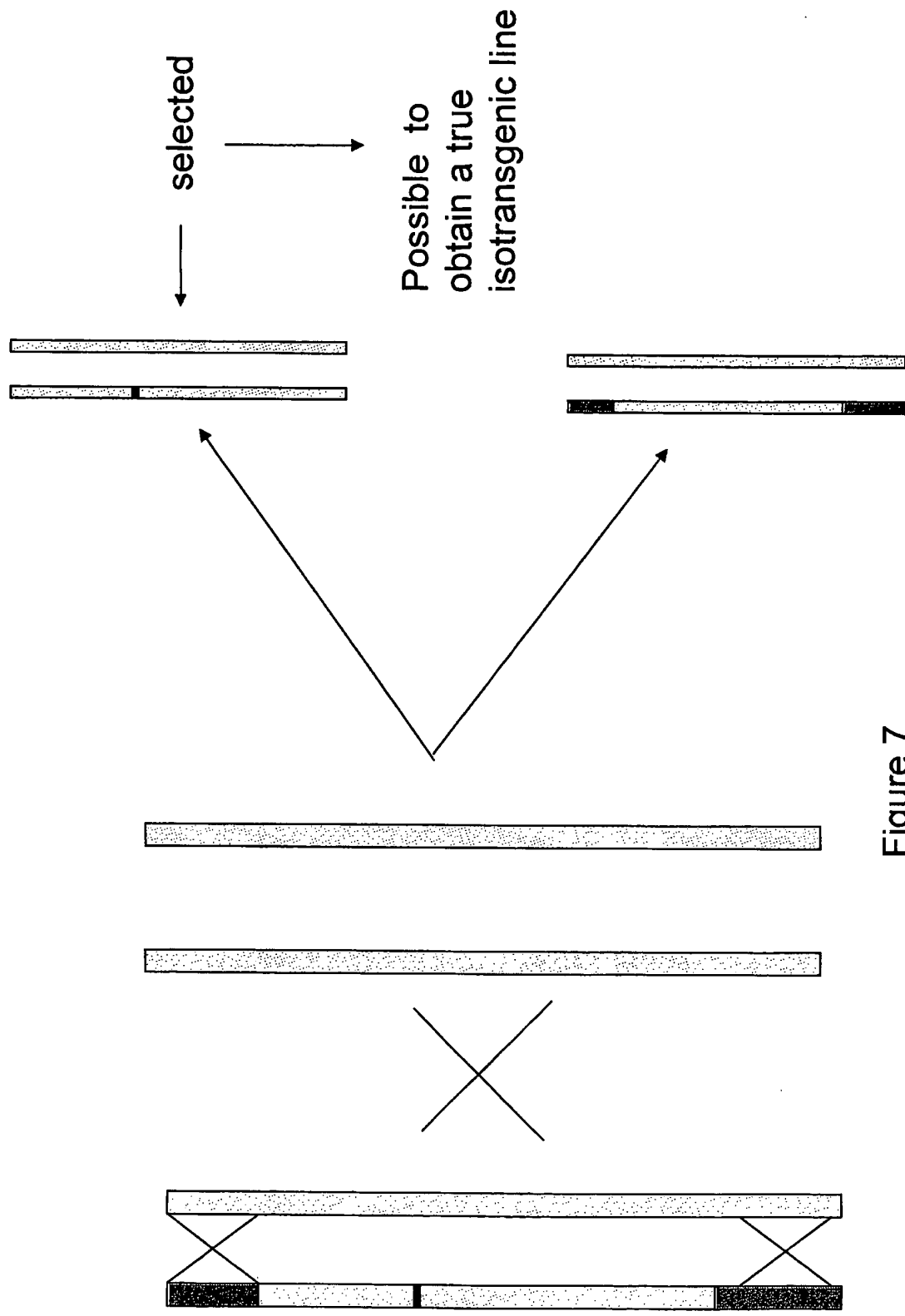


Figure 7  
Method according to the invention